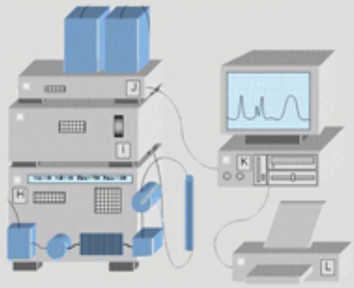
DO YOU KNOW WHAT REALLY GOES INTO RUNNING A HPLC?

HPLC SIMPLIFIED

★ A GUIDE ★

Anyone who is working in a laboratory is likely to use a HPLC at some time or the other. Right from the time when you complete your graduation, post graduation, doctorate or you are working in the industry, you may have to rely on HPLC to answer some vital questions. Most of us believe that it is enough to be able to run the software and inject samples. Far from this myth HPLC operation requires an understanding and knowledge of lot more than just the software and the hardware; these are in fact a very small part of the bigger picture. On an average day in a HPLC lab you would spend only a fraction of your time running the system. Most of the time will be spent preparing the samples, mobile phase, buffers, filling log books and various other forms and formats.

STEP # 1 **STARTING FROM THE START**



WHAT IS THE PURITY OF MY SAMPLE ?
WHAT ARE THE INGREDIENTS OF MY SAMPLE?
IS THE MATERIAL SUITABLE FOR MY APPLICATION?

3 KEY

1 FUNDAMENTALS OF ANALYTICAL CHEMISTRY

2 INSTRUMENT OPERATION

3 GOOD LABORATORY PRACTICES

THE SOLUTIONS OFFERED BY HPLC CAN BE BROADLY DIVIDED INTO THE FOLLOWING CATEGORIES

1. Establishing the identity
2. Determining the purity
3. Elucidating the composition
4. Isolation of components
5. Purification of components

THIS KNOWLEDGE OF HPLC IS VALUABLE IF YOU WORK IN ANY OF THESE FIELDS OF SCIENCE ▶▶ Chemistry · Agriculture · Environment · Bio sciences · Food · Pharmaceuticals

HERE'S GETTING THE MOST OUT FROM AN HPLC

THE HOW-TO GUIDE 18 EASY STEPS IN ALL

1 Laboratory Safety
safety always comes first

You must protect yourself, your colleagues and the environment.

Familiarize with hazard signs and use of safety gear.

Understanding storage and handling of materials and spill management.

2 Time Management
if you fail to plan you are planning to fail

Simple time management tools will help you make the most of your day.

A few minutes each day spent planning can enhance productivity many folds.

3 Finding and selecting the right method

First thing you need is a suitable method for analysing a sample.

This could be a STP or you might need to search for a method.

You might even have to develop a method.

A literature search can help find a suitable method.

4 HPLC – understand how it works to make it work for you

Understanding of the basic principles and system modules is essential.

Makes your work more enjoyable and you will get a better grasp of system operation

You will also be able to troubleshoot

5 Method Validation
validate if you need to

The method you use might have to be validated depending on the environment you are working in.

A complex method which is validated might be a better choice than a simpler un-validated method enhances your productivity many folds.

6 Buying, Qualifying and Calibrating the HPLC

Consider your prime requirements, reputation of the supplier to provide you efficient after sales maintenance and technical support

Duly qualify the new system including IQ, OQ and PQ before you start using it

Ensure the reliability of the instrument by regular calibration

7 Sourcing the supplies –
Chemicals, Standards, Columns

You require chemicals, reagents, solvents, columns etc . These might be available in your lab or you might need to source them

Purchase department might be non-technical and won't be able to source right material

Knowledge of sourcing materials can expedite worK

8 Sample Preparation –
undoubtedly the most critical step

It is usually the largest source of variation and can make or break your result

It can be single step dilution or a complex multistep procedure

Understand importance of each step to carry out analysis with accuracy and precision

9 Weighing – right every time

Weighing though seemingly very easy, is one of the most critical steps

Lot of mistakes happen during weighing

You need to be able to select the correct balance, perform and verify calibration

10 Volumetric Measurements –
vital for dilutions-only practice will make you perfect

Dilutions are critical in the preparation of samples, standards, mobile phases, buffers and derivatizing agents, etc.

Dilution often require several steps and error at any single step gets multiplied in subsequent . it is important to use calibrated volumetric glassware and micro pipettes .

Correct handling of micropipettes is essential to minimize errors.

11 System suitability

System suitability tests, are run before you start running samples.

Requires evaluation of parameters like theoretical plates, peak asymmetry, resolution, injector precision etc.

Only if these system suitability requirements are met can you proceed with the analysis

12 Standards –
sourcing, handling, need and traceability

Must be run to compare samples and calculate results

Their sourcing, handling, storage, preparation dilutions are critical to the analysis

Must be traceable to SI units or national or international standards

13 Quality control samples

Quality control samples serve as a double check to ensure the accuracy of results

These are certified samples having known concentration of the analyte

Usually have acceptance criteria for the deviation from the nominal value

14 HPLC software –
control, run, acquire, process and report

Software is used to set up a method, acquire data, define sequence in which samples and standard are to be injected

15 Preventive maintenance and trouble shooting

Things will go wrong. You need to be able to troubleshoot problems with the hardware, software, sample preparation and chromatography

Preventive maintenance plans reduce unexpected breakdowns are problems

16 Did the sample pass or fail?

This is the final question on all the data and efforts .

You should be able to interpret the results.

You should know the relevant specifications for the sample.

17 GLP, GDP and QMS –
why do we need all these documents?

The sheer size of documents and requirements might scare you.

Proper documentation is essential for authenticity of results.

It helps minimize errors and actually improves productivity

18 Laboratory Information Management Systems (LIMS)
– automate your laboratory operations

Most labs use some form of a LIMS.

Understanding it is essential for working in a modern laboratory

It improves increases throughput and the quality of your results

Helps manage your laboratory functioning with better planning

Authored by: Dr Saurabh Arora